

Role of Ribonucleic Acid Polymerase in Gene Selection in Procaryotes

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INTRODUCTION

There is ample evidence for gene selection during morphological development in procaryotes. The best examples include the sequential transcription of genes specific for phage replication and for bacterial sporulation. Although genetic and biochemical studies have suggested that a number of mechanisms may be involved in regulating gene expression, this review will assess the role that ribonucleic acid (RNA) polymerase (RPase) itself plays in the gene selection process.

The essential questions to ask are: (i) are there regulatory mechanisms that allow the RPase to play an active role in recognition of promoter sites during morphological development, and (ii) are these mechanisms, if they exist, different from those utilized during normal growth and during transition from one growth medium to another when no morphological changes are evident? One reason to suggest that the selectivity process (21) (the process that determines initiation and termination of transcription at specific sites) may differ during procaryotic development is the fact that a relatively small number of genes are being selected for sequential expression, which leads to an irreversible morphological change. In the case of normal phage and spore development, the sequence of events leads in fact to lysis and death of the host or mother cell, with the release of phage particles or a mature spore. This commitment to morphological development once initiated is not reversed. Therefore,

the regulatory processes that direct the transcription of developmental genes could differ from mechanisms involved in regulating transcription of genes involved in readily reversible physiological functions. A higher degree of specificity might, in fact, be necessary in this selective mechanism, since the cell is being committed to an irreversible morphological state, i.e., lysis.

From our current understanding of gene transcription (21, 22, 113), the RPase recognizes certain base sequences in deoxyribonucleic acid (DNA) (recognition site), binds to the DNA (binding site), initiates RNA synthesis (initiation site), elongates the RNA chain, and finally terminates RNA synthesis (termination site) with the release of a new RNA chain. The recognition-binding-initiation region for RPase is called the promoter site. Although there are compositional similarities between promoter sites (see below), the promoters recognized by the *Escherichia coli* RPase holoenzyme differ significantly in actual base sequences. This heterogeneity of the promoter regions suggests that different promoters may have different degrees of affinity for the RPase molecule; i.e., they represent slightly different substrates because of base sequence differences. These differences, if they are large enough, could determine whether a significant association can occur between that promoter region and RPase, whether a gene is transcribed slowly or rapidly, or whether a gene is transcribed at all. The promoter region is actually more than just a

binding site for RPase, since it also contains information for the binding of accessory regulatory factors such as the cyclic adenosine 5'-monophosphate-catabolite gene-activator protein (cAMP-CAP protein) complex (37), which will be discussed later. These accessory factors may act by destabilizing the DNA in the promoter region and facilitating the binding of RPase (41). The promoter region also may overlap repressor-binding or operator sites (10, 123).

Several possible mechanisms exist for the regulation of expression of developmental genes that are not expressed ordinarily during growth. If we assume that the promoter sites of developmental and vegetative genes are similar and can be recognized by the vegetative holoenzyme with equal efficiency, then the derepression of the developmental genes must be due to one of many possible mechanisms requiring an accessory factor that primarily determines whether a gene is expressed. For instance, if transcription of the developmental gene is controlled by a repressor mechanism, the repressor in the absence of a "developmental" inducer may either block the attachment of the RPase to the promoter site or prevent the progress of a bound RPase to the initiation site. The expression of this gene would require the presence of a "developmental" inducer to unblock the operator site. If a positive factor such as the *araC* product (213) or cAMP-CAP complex (37) is required to facilitate the attachment of RPase to the promoter site, then the controlled synthesis or presence of the necessary effectors would determine the gene selection process. If the expression of a developmental gene is dependent on a promoter of an adjacent operon, its expression would be dependent on overriding the termination signal of the adjacent operon; i.e., for transcription of the developmental gene to occur, an "antiterminator" factor would have to be produced to prevent the action of the transcription termination apparatus and allow the RPase to proceed from the adjacent operon to the developmental gene (164, 176). In a similar fashion, those biosynthetic enzymes that are derepressed to a higher degree during differentiation may have attenuator sites (11) that must be modulated by factors to allow a greater number of RPase molecules to proceed from the regulatory region to the structural genes. Thus, if one assumes that promoter sites for vegetative and developmental genes are similar and can be recognized by the vegetative RPase, then the transcription of developmental genes would be regulated primarily by the developmental accessory factors that control initiation and termination of tran-

scription. The vegetative RPase would be guided to the promoter regions of developmental genes by these developmental factors, and the RPase would be a secondary factor in the gene selection process.

On the other hand, it is possible that developmental genes have promoter regions that are significantly different in base sequence from the promoter regions of the vegetative genes. This difference in promoters may be sufficient to prevent their recognition by the vegetative holoenzyme and/or regulatory factors during growth. The expression of these genes would require (i) the synthesis of a completely new RPase molecule with a different recognition specificity, (ii) the modification of existent RPase molecules which alters their recognition specificity sufficiently to allow binding to these new promoter sequences, or (iii) the synthesis of new positive regulatory factors that only recognize promoter regions for developmental genes and facilitate the binding of existent vegetative RPase or modified RPase to these promoters. Thus, in the case of highly different developmental promoter regions, the selectivity process might require the use of modified or new RPase and/or new accessory factors.

The purpose of this review is to determine whether the current data support the hypothesis that RPase structural modification plays a critical role in gene selection during phage replication and bacterial sporulation.

PROPERTIES OF PROCARYOTIC RPase

Subunit Structure

The procaryotic vegetative-cell RPase consists of four major types of subunits, which have been named α , σ , β , and β' and are present in the molar ratio of 2:1:1:1 (18). The enzyme can exist as the holoenzyme ($\alpha_2\beta\beta'\sigma$) or as the core enzyme ($\alpha_2\beta\beta'$). Other polypeptides have been found to be associated with the holoenzyme or the core, e.g., ω with *E. coli* holoenzyme (18), but are not necessary for enzymatic activity. By the usual methods of purification, about 30 to 40% of the enzyme is found in holoenzyme form, and the rest is in the core enzyme form. However, since σ factor can dissociate relatively easily from the core (20), it is possible that some σ and other factors may be dissociated from the core during the purification process. Reference 19 contains a list of the subunits from several procaryotic organisms and their molecular weights. A significant difference in molecular weights is noted for the sigma factor from the various organisms. It appears, however, that the various sigma factors fall into two molecu-

lar-weight classes of about 90,000 and 55,000. There is also a variation in the molecular weights of the large β subunits, which range from 130,000 to 170,000 (19). The β and β' subunits are two of the largest polypeptides found in procaryotic cells, and this facilitates their identification in crude extracts (125).

Functions of the Subunits

The large subunits, although similar in molecular weight, differ in function (221). The β subunit binds rifampin, a potent inhibitor of RPase (154). Rifampin-resistant mutants have an altered β subunit that no longer binds rifampin effectively. Since rifampin inhibits the initiation step in RNA synthesis (79), the β subunit is probably involved in the initial catalytic steps and may be the catalytic component of the enzyme. The function of the β' subunit appears to be in binding DNA, since the RPase from a temperature-sensitive mutant with a mutation in the β' subunit binds less effectively to DNA *in vitro* at the nonpermissive temperature (146). Also, the partial proteolytic cleavage of the β' subunit leaves the other subunits and the core structure intact, but reduces the binding ability of the enzyme to DNA (103). Although the affinity of the enzyme for the DNA has been reduced by the modification of the β' subunit, the enzyme is still catalytically functional.

The sigma factor is necessary for initiation of transcription by the enzyme at the proper site on the DNA, i.e., at the promoter site. For instance, the sigma factor restricts initiation to the promoter sites of only pre-early T4 genes (8), at certain sites on the sense strand of fd and ϕ 80 phage DNA, resulting in RNA chains with specific purine nucleotide-initiated base sequences at their 5' ends (144, 192) and at sites in the DNA with very high association constants, e.g., $K_{\text{assoc}} = 10^{12}$ to 10^{14} M^{-1} (78). Sigma factor acts catalytically, since it dissociates from the core after the initiation process has been completed (207). This results in a sigma cycle in which the free sigma factor reassociates with another core enzyme to form a holoenzyme that is now able to initiate another RNA chain.

A new sigma factor designated σ' has been reported in *E. coli* (62). Its molecular weight is 56,000, smaller than that for sigma (90,000). Since anti- σ antibody does not cross-react with σ' , it is likely that σ' is not a proteolytic cleavage product of σ . The holoenzyme containing σ' (holoenzyme II) acts similarly to σ -containing holoenzyme (holoenzyme I) in that it transcribes phage DNA very efficiently in contrast to the core enzyme. Holoenzyme II differed

from holoenzyme I in that only holoenzyme II synthesized polyadenylate-polyuridyate in the absence of a DNA template, and only holoenzyme II utilized double-stranded DNA to form polyadenylate (87). The specific function and relationship of σ' to σ are still unknown.

The role for the α subunit has been difficult to define. It is an essential component of the enzyme, since subunit reassociation studies have shown that the presence of α subunits is essential for reconstitution of an active enzyme (14). A role in promoter recognition has been implied for the α subunit, since reconstitution studies with T4-modified α subunit have indicated that *E. coli* promoter recognition is greatly reduced when T4-modified α subunit is used (119). No information is available about the possible functions for ω subunit, which is associated with *E. coli* holoenzyme. Since reconstituted enzymes are active without ω polypeptide (76), it may have some as yet undefined regulatory role.

Although the holoenzyme is essential for proper initiation of transcription, the core can carry out the catalytic function, since only the core is found in the DNA-RNA-RPase elongation complex (16). Also in reconstitution experiments, the catalytic activity can be restored by reassociating only the core subunits (76, 85, 216).

In the subsequent analysis of the role of RPase for gene selection, particular attention will be paid to determine whether any covalent modifications of the subunits of the holoenzymes, any new sigma-like factor, or a new RPase molecule is formed which directs the enzyme to a promoter site for a developmental gene.

Genetics of the RPase Subunits

Since the RPase is comprised of a number of subunits and the regulation of their synthesis may play a role in gene selection, it is proper at this time to summarize the information available as to the genetic loci of the subunits and the regulation of the synthesis of the subunits and the core enzyme (172).

The genetic loci of the core subunits have been determined for *E. coli*. The genes for β and β' are located between the *bfe* and *purD* loci at 89 min on the *E. coli* map (6, 219). The genetic locus for the α subunit is quite distant from the loci for the β subunits and is located between the *spcA* and *trkA* loci at 72 min on the *E. coli* map. It is found within a cluster of ribosomal protein genes and is coordinately expressed and possibly cotranscribed with the genes for ribosomal proteins S11, S4, and L17

(90). The expression of the α gene is probably also under stringent control (38). The genetic locus of sigma factor is still unknown.

The genes for β and β' are located in the same operon with the order $p\text{-}\beta\text{-}\beta'$ (where p stands for promoter) and are transcribed in a clockwise order (56). The β and β' subunits are coordinately synthesized, and there is some but not complete coordination of their synthesis with that of the α subunit. During nutritional shiftup and shiftdown, the differential rate of synthesis of α , β , and β' increased and decreased, respectively, leading to an increase or decrease in the rate of synthesis of core enzyme (33, 88, 89, 141). These shifts in rates of synthesis were somewhat similar to those found for ribosomal proteins. However, these studies have shown that there is always an excess of RPase molecules present in the cell, the rate of growth is not dependent on the rate of synthesis of the core, and the growth rate is not directly related to the concentration of RPase in the cell except near the maximum rates of growth. For instance, although the growth rate increased by a factor of 5, the increase in the differential rate of synthesis of RPase increased by only a factor of 1.5. Although there is a coupled synthesis of the β subunits with α subunit, their occurrence in different operons results in different rates of synthesis. The ratio of α to β' synthesis has been found to be about 5:1 (54). Therefore, the coordination of the synthesis of the core subunits is not exact, and α synthesis is regulated independently.

In terms of the overall regulation of synthesis of the enzyme, a control mechanism, as yet undefined, exists which regulates the minimum concentration of core in the cell (56). The regulatory mechanism is such that an increase in gene dosage does not result in a sudden increase in the overall rate of core synthesis (94). Evidence has been obtained for the positive regulation of the synthesis of RPase (132). A gene located between *polA* and *rha* is necessary for RPase core synthesis. A mutation in this (*Am*)100 gene was suppressed by amber suppressors. Although the product of this gene was necessary for the expression of the α and β subunit genes, it did not control the synthesis of σ or ρ factor.

A number of experiments have suggested that the expression of the β genes may be autoregulated (67, 75). The transient inhibition of RNA polymerase activity by rifampin in heteromerodiploids (e.g., *rif^r/rif^s*) results in an increased rate of synthesis of the β subunits (53, 67, 75, 134, 199, 200). A poor rate of translation of the β messenger RNA (mRNA) also leads to

an induction of the β subunits (67). However, other data indicate that a greater complexity exists than a simple autoregulation by β subunits or by an inactivated enzyme. An excess of β and β' subunits in itself did not reduce the rate of their own synthesis (198). In a mutant with a temperature-sensitive mutation in the β subunit gene, the nonpermissive temperature resulted in a two- to threefold decrease in the rate of synthesis of both β subunits (95). On the other hand, a temperature-sensitive mutation in the β' gene resulted in a five- to sixfold increase in the rate of synthesis of the β subunits at the nonpermissive temperature (95). The results with the temperature-sensitive mutants (95) and with streptolydigin (134), an inhibitor of RPase during the elongation step, suggest that an inactive enzyme in itself does not lead to derepression; one consistency noted, however, was that a temperature-sensitive mutation in the β' gene usually resulted in an increased rate of β subunits (42, 95). The specific conformation resulting from the β' mutation may lead to release from autoregulated repression.

The effect of rifampin on the transient stimulation of RPase subunit synthesis is also more complicated than originally believed (134, 199). The extent of derepression of the different subunits depended upon the strain of bacteria and the concentration of rifampin used. When the wild-type haploid strain was treated with 50 μg of rifampin per ml, a twofold stimulation of synthesis of the core subunits was obtained, whereas an eightfold stimulation of sigma factor was observed (134). With a strain partially resistant to rifampin (126) (i.e., resistant at a high concentration [500 $\mu\text{g}/\text{ml}$]), a transient and striking stimulation of only the β' and σ subunits was noted. These and the previously discussed results suggest that a specific effect of rifampin, but not the inactivation of the enzyme or the β subunit per se, is involved in the transient stimulation of core subunit synthesis and that the specific conformation of the enzyme resulting from the rifampin-core interaction could lead to release from autoregulation repression.

In all the studies there is good evidence that the regulation of the synthesis of sigma factor is independent from that for the core subunits (53, 54, 89, 134). Sigma factor synthesis is not coordinated with either α or $\beta\text{-}\beta'$ and occurs under usual growth conditions at about 0.3 to 0.4 times the amount of core subunits (54, 89). Sigma synthesis is under the control of at least three distinct genetic loci, with one located very close to *rpo* and the other two at the *argH-bfe*

and *metB* regions of the *E. coli* map (133). Sigma is not controlled by the positive regulatory mechanism that controls the synthesis of the core subunits (132).

In similar analyses with the transcription termination factor rho (162), rho synthesis was not dependent on the positive control that regulated the core subunits (132). Furthermore, the regulation of rho synthesis was distinct from α synthesis and resembled that reported for sigma synthesis (14, 132). Since sigma and rho are cycled onto and off of RPase rapidly and are not present in stoichiometric amounts with the core, the control of their synthesis could be regulated independently from that for the core subunits. The *rho* gene has been mapped at 83 min near *ilv* on the *E. coli* map (34, 84, 159). Most interestingly, there is strong evidence that the *suA* polarity-suppressing gene is identical with the *rho* gene (158, 160). Missense mutations in *suA* result in structurally modified rho factor (158).

In summary, the syntheses of β and β' are tightly coordinated, and some of the mechanisms that control their synthesis also appear to regulate α synthesis, but with distinct differences. The regulation and synthesis of σ and ρ factors is clearly distinct from that for the core subunits.

Accessory Regulatory Factors

In addition to the sigma and rho factors, which regulate initiation and termination of transcription, several accessory factors regulating RPase activity have been well characterized. These factors generally exert their effects by interacting with the DNA rather than with the RPase. In any consideration of gene selectivity, these factors would have to be considered. They include negative factors or repressors whose physical presence on the DNA prevents the RPase from initiating transcription and positive factors whose presence is essential for initiation of transcription by the RPase (21).

The repressors are proteins that bind to specific operator sites on the DNA and block transcription by RNA RPase. In the *trp* operon of *E. coli*, the operator and promoter sites are so located that the binding of the tryptophan-repressor complex to the DNA site prevents the binding of RPase to its site and vice versa (10). Therefore, the promoter and operator sites appear to overlap. In the *trp* operon, the expression of the genes is prevented when a high level of tryptophan is available, since tryptophan binds to the repressor to form a tryptophan-repressor complex that has a high affinity for the operator site (166, 181). When tryptophan

concentration is low, the free repressor does not bind to the operator, thus allowing transcription to occur. The regulation of this operon is also more finely regulated by the presence of an attenuator locus some 130 bases distal from the operator site (11, 12, 104). This locus is in the *trp* leader mRNA region, which is a 160-base-long segment of RNA that precedes the initial codon for the first polypeptide in this polycistronic mRNA. When the level of tryptophan is moderately high, some mRNA's may be initiated, but only about one in ten RPase molecules will proceed beyond the attenuator point, which is actually a regulatory termination point. At this site, which appears to be controlled by the rho termination factor and tryptophanyl-transfer RNA (tRNA) synthetase (13,130), transcription can be prematurely terminated with the release of a short 130-base piece of RNA. It has been suggested that the concentration of tryptophanyl-tRNA and its interaction with rho factor determines whether the RPase proceeds beyond this point to the mRNA sequence for the first structural gene or whether transcription is terminated at this attenuator locus. Attenuator-like loci may also regulate the *his* (3, 92) and *ilv* (211) operons and lambda functions (162, 163, 168).

With the *lac* operon, earlier studies suggested that the *lac* repressor and the RPase could bind to their sites simultaneously; i.e., the presence of the *lac* repressor on the operator site appeared not to prevent RPase from binding to its promoter site (37). More recent results (121) with the *lac* operon indicate that there is a functional overlap of the operator and promoter sites and that the presence of repressor prevents RPase from proceeding beyond this point to the structural genes. This is analogous to the *trp* locus control. When a suitable inducer molecule binds to the *lac* repressor to form an inducer-repressor complex, this complex has a much reduced affinity to the DNA and is dissociated from the DNA, thus allowing the RPase to proceed to the structural genes. However, it has been shown that the *lac* locus is also controlled by a positive regulatory factor or a catabolite gene-activator protein which has been called either the CAP (catabolite gene-activator protein) (223) or CRP (cAMP receptor protein) (52) protein.

To have expression of the *lac* operon, catabolite repression must be released, and during this process cAMP is formed from adenosine 5'-triphosphate by adenylate cyclase. When cAMP associates with the CAP protein, the cAMP-CAP protein complex is able to bind to the *lac* promoter region (121). This association

of the CAP protein to the DNA apparently causes a destabilization of the DNA, which facilitates the binding of the RPase to the promoter region and the formation of an open complex (41) that is necessary for the initiation of RNA synthesis. Thus, for the full expression of the *lac* operon, the presence of cAMP, CAP, inducer, and RPase is essential; both a positive and a negative mode of regulation are operative. This type of control (135) is believed to be a major control mechanism for catabolite-repressed genes (147, 161).

Another aspect of this type of regulation is illustrated with the arabinose locus. The *ara* locus, which codes for the enzymes for arabinose utilization, is regulated by arabinose, an inducer-effector, which converts the repressor form of the product of the regulatory gene, *araC*, to the activator form (105). This activator form is essential for initiating transcription by the RPase. However, in addition to the presence of arabinose and the *araC* product, cAMP and CAP protein are also required for the full expression of the *ara* operon (222). Thus, in this system two positive regulatory proteins, *araC* product and CAP, are required for RPase activity.

A number of other protein factors have been reported that stimulate RPase (28, 29, 65, 101, 117, 131, 156, 169). These factors could be increasing the initial start frequency, augmenting the elongation rate, decreasing the frequency of chain termination, freeing terminated enzymes from the DNA for restarts, or rescuing RPase from abortive termination. Usually these factors fall into two classes: (i) those that bind to DNA and facilitate RNA synthesis (28, 29, 65, 169), and (ii) those that interact with the RPase to stimulate RNA synthesis (156). Of the factors that have been reasonably well characterized, most increase the frequency of initiation. Several of the stimulatory factors are purified from the ribosome fraction (101) (i.e., they appear to have high affinity for ribosomes *in vitro*), whereas others are found in the supernatant fraction (65, 131). The significance of the ribosome association *in vivo* is still unclear.

The DNA-binding proteins could either activate promoter sites, resulting in greater initiation rates, and/or inactivate improper start sites and promote greater transcription specificity. The D (65), HU (169), and H₁ (28, 29) factors exert their maximum stimulatory effect on RPase holoenzyme when there is a stoichiometric weight ratio of the protein to the DNA. The D protein raises the T_m of the template DNA and increases the initial start frequency

and the specificity of transcription from the early genes of lambda phage DNA (65). Since the lambda repressor blocks the synthesis of D-factor-stimulated RNA, there appears to be a specific effect of the D factor on initiation of lambda DNA.

The H₁ factor binds to native DNA and has been found to stimulate synthesis of *lac* RNA from the $\phi 80d$ *lac* DNA more than lambda RNA from lambda DNA (28). Its stimulation of *lac* RNA synthesis (fivefold) was equal to or greater than the effect that cAMP-CAP protein had on stimulating transcription of the *lac* locus. The state of the promoter locus also had significant effects on the H₁ stimulation of *lac* RNA synthesis. If the expression of the *lac* locus was under control of the *trp* or the lambda promoter, the stimulation by H₁ factor was only twofold or about equal to the stimulation of lambda RNA synthesis from lambda DNA. A relationship between the effect of the H₁ factor and the cAMP-CAP effect is suggested by the fact that a promoter ($\phi 80d$ *lac* p_R *uv-5*) which is CAP insensitive is also insensitive to H₁ stimulation. In the $\phi 80d$ *lac* p_R *uv-5* mutant, the base sequence in the RPase-binding site is altered (66) in such a manner that RPase can initiate transcription in the absence of the cAMP-CAP. If the role of cAMP-CAP is to destabilize the RPase-binding site to facilitate RPase binding, the H₁ may be having a similar effect.

Although the data indicate that specificity of transcription is increased, they do not demonstrate unequivocally that these RPase-stimulating factors play a role in specific gene selection. Their effects on a broad number of templates indicate that they may play a general role in promoting initiation at the proper sites and preventing initiation at improper sites.

Recognition Sites on DNA for RPase and Accessory Regulatory Factors

One of the major factors to consider in analyzing the gene selectivity process is the information concerning the base sequences recognized by RPase. Recent advances in DNA- and RNA-sequencing techniques (41, 170) have allowed the determination of a number of DNA base sequences in critical regulatory areas for transcription. These analyses have permitted the identification of certain patterns, although much more information is required before any definitive conclusions can be drawn.

In Tables 1 and 2 are summarized a number of base sequences that are recognized by *E. coli* holoenzyme for the initiation of transcription. Three types of sites have been established: (i) a recognition or "R" site, which is initially recog-

TABLE 1. Initial Recognition Site for *E. coli* RPase^a

DNA	R site	B site	I site	References
Lac wild type	AGGCACCCCAGGC <u>TTTACACTTTAT</u> GCTTCCGGCTCG	<u>TATGTTG</u>	<u>TGTGGA</u>	41
Lambda p _R	TAACACCGTGCGTG <u>TTGACTATTTTA</u> CCTCTGGCGGT	<u>GATAATG</u>	<u>GTTGCA</u>	123, 124, 150, 208
Lambda p _L	TATCTCTGGCGGTG <u>TTGACATAAATA</u> CCACTGGCGGT	<u>GATACTG</u>	<u>AGCACA</u>	123, 124, 150, 208
Lambda p _{rm}	CAACACGCACGGTG <u>TTAGATATTAT</u> CCCTTGGCGGT	<u>GATAGAT</u>	<u>TTAACGTA</u>	142
Simian virus 40	GAATGCAATTGTTG <u>TTGTTAACTTGTTTATT</u> GCAGCT	<u>TATAATG</u>	<u>GTTACA</u>	39

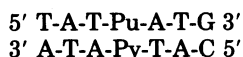
^a R site is the recognition site; B site is the tight binding site; I site is the initiation site (the underlined letter represents the 5' end of the transcribed mRNA).

TABLE 2. Binding sites for *E. coli* RPase^a

DNA	B site	I site	References
T7 A1	GAAGTAACATGCAG <u>TAAGATA</u>	CAAATCGCTAGGTAACACTAGCAGT	151
T7 A3	GAAGTAAACACGG <u>TACGATG</u>	TACCACATGAAACGACAGTGAGTCA	151
fd G2	GCTTTGCTTCTGAC <u>TATAATA</u>	GACAGGGTAAAGACCTGATTTTTGA	175, 196
fd G3	TGTTTCGCGCTTG <u>TATAATC</u>	GCTGGGGTCAAAGATGAGTGTT	196
Lambda p _R	TTACCTCTGGCGGT <u>GATAATG</u>	GTTGCATGTACTAAGGAGGTTG	123
Lambda p _L	ATACCACTGGCGGT <u>GATACTG</u>	AGCACATCAGCAGGACGCACTGAC	123
Lac wild type	ATGCTTCCGGCTCG <u>TATGTTG</u>	TGTGGAATTGTGAGCGGATAACAA	41
Lac UV-5	ATGCTTCCGGCTCG <u>TATAATG</u>	TGTGGAATTGTGAGCGGATAACAA	66
tRNA ^{Trp}	GGCGCGTCATTTGA <u>TATGATG</u>	CGCCCCGCTTCCCGAT	180
Trp	ATCATCGAAGTAGT <u>TAAGTAG</u>	TACGCAAGTTCACGTAAAAAGGGT	10
Simian virus 40	TGTTTATTGCAGCT <u>TATAATG</u>	GTTACAATAAAGCAATAGCA	39

^a B site is the tight binding site; I site is the initiation site (the underlined letter represents the 5' end of the mRNA).

nized by the RPase; (ii) a tight binding or "B" site, which permits the RPase to associate tightly with the DNA; and (iii) the transcription initiation or "I" site, which is transcribed into the terminal 5'-nucleotide of the product RNA. The R site is generally rich in A-T base pairs (Table 1) and is located about 25 to 35 nucleotides on the 5' side of the I site. In the four R sites that have been sequenced (Table 1), there are significant differences in base sequence, indicating that the *E. coli* holoenzyme can recognize similar, but not identical, base sequences. After recognition of the R site, the enzyme moves in the 3' direction to the B site (Table 2), which is seven base pairs long and has the following general sequence (66, 151):



This type of sequence has been found for all B sites, with only slight variations seen with lambda phage and *E. coli* trp B sites. The B site is usually four to seven base pairs on the 5' side of the I site. The RPase can bind and protect about 30 base pairs of DNA from deoxyribonuclease activity; the RPase can, therefore, be in contact with the B and I sites simultaneously.

The R site is required for the initial recogni-

tion of a specific transcription site, since mutations in the R region prevent transcription in vivo (66), and RPase in vitro cannot use a DNA fragment missing the R region for a template. The R site may be the most critical site for the gene selectivity process, since the initial recognition of the gene to be transcribed is at this site.

The B site, which is also rich in A-T base pairs and crucial for tight binding of the RPase to DNA, may play a role in facilitating the initiation of transcription. When two base pairs were altered in the lac B site (see lines 7 and 8, Fig. 2), converting wild type to UV-5, it was found that transcription could be initiated in vitro with UV-5 DNA in the absence of the cAMP-CAP complex. This suggests that the B site sequence in the wild type, which has an additional G-C base pair, is more stable, and that expression of wild-type lac requires the cAMP-CAP complex to destabilize the region and allow the tight binding of the enzyme to the B site.

The sequence analyses of transcription products have revealed patterns suggesting that A-T base pair regions may be involved in the termination signal. A sequence of six to seven adenylate residues in DNA is transcribed into

short polyuridylylate sequences near or at the 3' end of mRNA's (Table 3). In addition, the final nucleotides at the 3' end of the mRNA are usually -U-U-A, -U-A-U, -U-A-A, or -U-U.

In addition to the high A-T base pair content of initiation and termination regions, one other interesting observation has been made concerning regulatory sites that are recognized by accessory factors. In sites recognized by repressors, cAMP-CAP protein complex, and termination factors, there are base sequences with twofold rotational symmetry (Table 4). Also, in some sites recognized by RPase, there are symmetrical base sequences, e.g., for *E. coli trp*, fd phage, and *E. coli* tRNA^{trp} promoters. In *E. coli lac*, there appears to be a region that lacks symmetry and binds RPase (41); however, even in this case there are regions of symmetry very close to the postulated RPase site. Although the significance of these sequences is still unknown, their unfailing presence to date at these regulatory sites suggests a role as a recognition site for the accessory protein factors.

The significance of these findings to our discussion is the fact that *E. coli* holoenzyme appears to be able to recognize and bind to a number of similar, but distinctly different, sequences of bases in the promoter region. The association constant of the holoenzyme with each sequence could regulate the rate of initiation of transcription. Therefore, one should find a range of initiation rates dependent on the relative association constants. Obviously, an accessory regulatory factor could also affect the association constant and, therefore, the initiation rate. A question of prime interest is, "Are there recognition or binding sequences for developmental genes which are so different in base sequence that they are not recognized by vegetative holoenzyme and which require a modification of the RPase molecule in order for proper recognition to occur?"

In an attempt to answer this question, studies on phage development in *E. coli* and *Bacillus subtilis* and on sporulation in *B. subtilis* have been analyzed to determine the gene selectivity function of RPase.

RPase ROLE IN PROCARYOTIC DEVELOPMENTAL SYSTEMS

T4 Phage Development

The analysis of the development of T4 phage in *E. coli* provided the earliest evidence for selective gene transcription by showing that a temporal sequence of transcription products occurred (171, 214). It was obviously of interest to determine how the temporal occurrence of promoter recognition occurred in this system. For this review, evidence will be discussed to see whether any modification of RPase structure per se plays a direct role in T4 promoter recognition. Furthermore, is there evidence that T4 promoters are different from *E. coli* promoters? A brief discussion of the evidence and current understanding of T4 phage DNA transcription will provide a background for my discussion of the role of RPase in this process. Concise reviews of the regulation of transcription during T4 infection have been presented (214).

After T4 infection, there is a sequential appearance of gene products that have been defined by their appearance and homology to certain regions of the T4 genome by DNA-RNA hybridization analyses. The early or prereplicative RNAs are made before phage DNA replication, whereas the late or postreplicative RNAs are made only after the onset of DNA replication, which starts at about 5 to 6 min after infection at 30°C. The early gene products are complementary only to the 1 strand (171), whereas many of the late gene products are also complementary to the r strand of T4 DNA (73).

The prereplicative or early period (E) is di-

TABLE 3. Sequences of bases in the RNA at the 3' terminus

RNA	Condition	3' Terminus of RNA	References
Lambda 6S RNA	-rho in vitro	-A-U-U-U-U-U-U-A	31, 102, 168
Lambda 4S RNA	-rho in vitro	-U-U-U-U-U-U-A	31, 32, 168
Lambda 6S RNA	+rho in vitro	-U-U-U-U-U-U-A-U	31, 168
Lambda 4S RNA	+rho in vitro	-U-U-U-U-U-U-A-U	31, 168
Lambda 6S RNA	In vivo	-U-U-U-U-U-U-A	184
Lambda 4S RNA	In vivo	-U-U-U-U-U-U-A	32
φ80 phage	In vivo	-C-U-U-U-U-U-A-A	149
T7 phage	In vivo and in vitro	-C-C-C-U-U-U-A-U	96, 167
<i>E. coli</i> small RNA	In vivo	-U-U-U-U-U-U-A	83
<i>E. coli</i> 6S RNA	In vivo	-A-U-U-C-(C, A)	17
<i>E. coli trp</i> leader	In vitro	-C-U-U-U-U-U-U-U or one more U	11
<i>E. coli trp</i> leader	In vivo	-C-U-U-U-U-U-U-U	11

TABLE 4. Regions with twofold base sequence symmetry in DNA^a

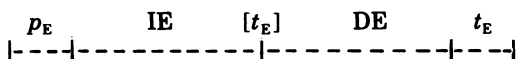
Region	References	
Operator regions		
<i>E. coli lac</i>	41	<div><div>I</div><div>TGTGTGGAATTGTGAGCGCATAACAATTTACACA</div><div>ACACACCTTAACACTCGCGTATTGTTAAAGTGTGT</div></div>
<i>E. coli trp</i>	10	<div><div>-----+-----I</div><div>CGAACTAGTTAACTAGTACGCAAGTTC</div><div>GCTTGATCAATTGATCATGCGTTCAAG</div><div>----- -----</div></div>
Lambda <i>o_R</i>	123	<div><div>TATCACCGCAAGGGATAAATATCTAACACCGT</div><div>ATAGTGGCGTTCCTATTATAGATTGTGGCA</div><div>GCGTGTGACTATTTTACCTCTGGCGGTGATAATGG</div><div>CGCACAACTGATAAAATGGAGACCGCCACTATTACC</div></div>
Lambda <i>o_L</i>	123	<div><div>I</div><div>TTGCATGTA</div><div>AACGTACAT</div><div>TATCTCTGGCGGTGTTGACATAAATACCACTGGCGGTGATA</div><div>ATAGAGACCGCCACAACGTATTTATGGTGACCGCCACTAT</div><div>I</div><div>CTGAGCACATGAG</div><div>GACTCGTGTACTC</div></div>
CAP binding region		
<i>E. coli lac</i>	41	<div><div>TGAGTTAGCTCACTCA</div><div>ACTCAATCGAGTGAGT</div></div>
Promoter regions		
<i>E. coli tRNA^{Tyr}</i>	180	<div><div>GGCGCGTCATTTGATATGATGCGCCCCG</div><div>CCGCGCAGTAAACTATACTACGCGGGGC</div><div>I</div></div>
fd phage G2	175, 196	<div><div>CTGACTATAATAGACAGGG</div><div>GACTGATATTATCTGTCCC</div><div>I</div></div>
fd phage G3	196	<div><div>GCGCTTGGTATAATCGCTG</div><div>CGCGAACCATATTAGCGAC</div><div>I</div></div>
Terminator regions		
<i>E. coli tRNA^{Tyr}</i>	112	<div><div>5' CCATCACTTTCAAAAGTCCCTGAACT</div><div>3' GGTAGTGAAAGTTTTTCAGGGACTTGA</div><div>-----</div></div>
<i>E. coli trp</i> attenuator	11	<div><div>GCCCCGCGGGCTTTTTTTTGAACAAAA</div><div>CGGGCGCCCGAAAAAAACTTGTTTT</div></div>

^a Symbols · and ° represent the center of symmetry.

vided into the immediate-early (IE), the delayed-early (DE), and quasi-late (QL) or middle transcription periods. The IE RNA is made within 0 to 1.5 min of infection and can occur in the absence of protein synthesis, i.e., in the presence of chloramphenicol (171). The DE RNA is made after 2 min of infection, and its synthesis is inhibited in cells that have been treated with chloramphenicol before infection. Earlier in vitro experiments had shown that holoenzyme from uninfected cells could utilize mature T4 DNA and transcribe both IE and DE

genes (127, 128) and that the presence of the termination factor rho allowed only IE transcription. This suggested the possibility of two types of models. One model assumed that an antiterminator factor was allowing the enzyme to proceed from the IE genes to the DE genes by overcoming the effects of a transcription termination site between the IE and DE genes (171, 176). The other model indicated that termination would be effected by rho factor for the transcription of the IE genes and that a new positive regulatory factor would allow the

RPase to recognize a new promoter site for DE genes (204, 205). These models were not mutually exclusive, since this hypothesis implied that an IE gene product acted either as an antiterminator or as a T4 sigma-like factor and allowed the transcription of the DE genes. More recent studies suggest the following. (i) An early promoter, designated p_E , in fact serves for both the IE and DE genes (142): the IE genes are proximal to p_E and DE genes are distal to p_E , thus causing a 2-min delay in the transcription of DE genes; most DE RNAs arise as distal portions of polycistronic mRNA's, which contain IE RNAs at the 5' end. (ii) No T4-encoded protein appears to be required for the transcription of either the IE or DE genes, since uninfected *E. coli* lysates can transcribe and translate both IE and DE genes in vitro (143, 197). The chloramphenicol effects noted earlier can perhaps be ascribed to the polar effects exerted by the antibiotic on the transcription process. An induced termination may be effected on a termination site lying between the IE and DE regions:



The $[t_E]$ may be affected by chloramphenicol treatment, resulting in premature termination of transcription in vivo. The t_E is the normal termination site in a normal transcriptional process.

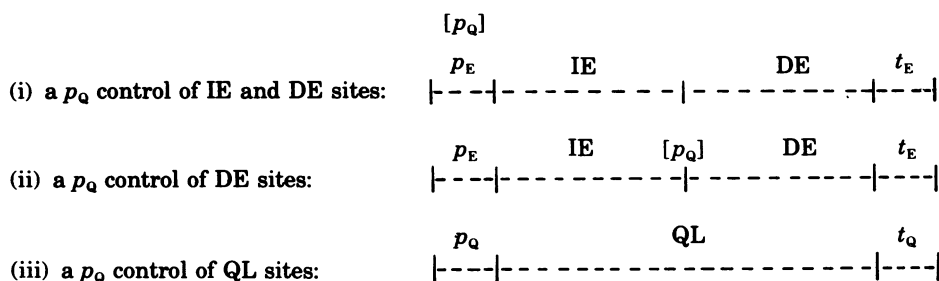
The QL RNAs are initially transcribed at about the same time as the DE RNAs, i.e., about 1.5 to 2.0 min after infection. The QL RNAs can be distinguished from DE RNAs in that they occur at low concentrations before DNA synthesis and at high relative abundance at the postreplicative period (171). Also, a temperature-sensitive G1 mutant is unable to synthesize QL RNA at the restrictive temperature. The mutation may act by blocking the capacity of the T4 DNA template to support QL RNA synthesis (197). Some of this third class of pre-replicative RNAs is not derived from the p_E promoter sites. The QL RNAs can be transcribed from either a p_E or a p_Q site or only from a p_Q site. The topology of the QL genes for these two cases is illustrated as follows (142):

In (i), some p_E promoters can also be controlled as p_Q sites, resulting in QL-type expression of some IE and DE sites. In (ii), the RPase can initiate at the p_E site and transcribe both the IE and DE regions. Under certain conditions, however, initiation can occur internally at the $[p_Q]$ site, and only the DE region will be transcribed. In this case, a DE region will be under the control of a $[p_Q]$ promoter and the transcript will be a QL product; e.g., the rIIB protein can be synthesized either as a DE or as a QL protein (30). In (iii) there are certain QL genes that are not recognized at all if p_Q recognition is blocked by rifampin (142). The transcription of these QL genes would be dependent on RPase that could recognize p_Q specifically. Thus the QL expression of genes is quite complex and may require some T4 gene-controlled functions that effect p_Q promoter recognition.

The existence of p_Q sites is supported by the following facts. (i) The transcription of rIIB RNA that is a DE RNA occurs initially at 2 min after infection at 30°C; it can also occur at the 5' end of the mRNA (176, 177) and therefore appears to be controlled also by p_Q site. (ii) Some QL RNA synthesis is initiated and becomes rifampin resistant 1.5 min after infection (142). (iii) Some p_Q -controlled protein synthesis is blocked completely when p_Q expression is blocked by rifampin (142, 197). (iv) The components required for recognition of p_Q promoters are missing in uninfected cells (142, 197).

A major factor that appears to affect this situation is the property of the T4 DNA after infection (197). Although mature T4 DNA cannot serve as the template for p_Q transcription in vitro, complementation studies in vitro indicated that the T4 DNA obtained from normally infected cells was transcribed at a p_Q promoter by RPase prepared from cells that had been treated before infection with chloramphenicol (197). These results indicate that the state of the DNA during the infection cycle may be the major factor determining p_Q promoter recognition.

The transcription of late (L) RNA during T4 infection occurs from about 9 to 10 min after the initiation of DNA replication at about 6 min



(171). The L genes are all expressed within a very short time of each other (140), suggesting that postreplicative genes can be considered as one temporal class or group of genes.

The transcription from prereplicative to postreplicative transcription is normally dependent on DNA synthesis and the protein products of genes 33 and 55 (69) and gene 45 (157, 213a). DNA "processing" (214), which makes the DNA competent for L-gene expression, may include changes in primary structure, e.g., endonucleolytic nicks, and in superhelix structure. These changes may occur at replication forks or other sites such as membrane-DNA interactions. Although cessation of DNA replication results in a selective reduction of L-RNA synthesis, continuing net accumulation of DNA does not appear to be essential; e.g., DNA ligase mutants turn over newly made DNA, but still allow late transcription (214). Even the effects of cessation of DNA synthesis can be relieved by preventing repair of DNA; a triple mutant with temperature-sensitive mutations in T4 DNA polymerase (*pol*⁻), T4 DNA ligase (*lig*⁻), and the exonucleolytic function (*exof*⁻) was able to form late protein at nonpermissive temperatures (214). It was proposed that late transcription units were inaccessible in intact mature DNA, but "open" replicating forms were accessible. Although this triple mutant could not successfully replicate DNA, its DNA was converted to an open form, perhaps endonucleolytically, and kept in that state by the mutations in the ligase and exonucleolytic functions. This nonreplicating but open form then allowed late transcription to occur.

The sequential transcription of IE, DE, QL, and L T4 genes suggests several mechanisms of regulation. The IE and DE genes can be transcribed by the host RPase. However, QL and L genes appear to require T4 gene functions before they can be expressed. One mechanism that has been proposed is that, after the IE genes have been expressed, one or more of the IE gene products "modifies" the host RPase. (Modification will be used in the broadest sense.) This modification changes the promoter specificity of the enzyme, allowing it to recognize QL genes or *p_Q* promoters. A product of the QL genes then modifies the RPase further to allow recognition of L-gene or *p_L* promoters in the open replicating DNA structure. Is there any evidence that modifications of RPase occur, and if they indeed do occur, do these modifications direct gene selectivity?

After T4 infection, a number of modifications of the host polymerase have been observed and will be discussed shortly. In addition, it has

been shown that the host β subunit continues to function throughout the phage replication cycle and that the host RPase molecules existent at the time of phage infection do not turn over during phage development (69). The RPase modifications that occur after T4 phage infection can be summarized as follows.

(i) **Alteration of host RPase.** A phage-coded enzyme that transfers an adenosine 5'-diphosphate (ADP)-ribosyl residue from nicotinamide adenine dinucleotide (oxidized) to specific arginine sites on proteins is carried in the head of mature T4 phage particles (68, 165, 173). Upon infection, the enzyme is injected into the host and transfers ADP-ribosyl residues to the α , β , β' , and σ subunits of host RPase. The α subunit reacts very specifically with the enzyme, and only one-half of the α subunits are labeled with ADP-ribose. This alteration of RPase subunits occurs within 2 min of infection, proceeds in the presence of high concentrations of chloramphenicol, and is independent of protein synthesis and expression of the phage genome (165).

(ii) **Modification of α subunits.** After alteration, another phage-coded enzyme different from the enzyme causing alteration of the host RPase also transfers adenosine diphosphoribose to specific arginine sites on both α subunits of host RPase (68, 173). The σ , β , and β' subunits are not modified by this enzyme. Modification is carried out by a QL enzyme synthesized about 3 to 7 min after infection (81). The α subunit remains modified throughout infection (200).

These alterations and modifications of the host RPase subunits do not appear to play an essential role in transcriptional specificity during T4 development, since T4 mutants of the *alt* and *mod* genotypes have been isolated which show completely normal development and the expression of all classes of T4 genes. The isolation of a double mutant *alt mod* should reveal absolutely whether ADP-ribosylation is required for normal phage development. The *mod* gene has been mapped in a clustered region of "nonessential" T4 genes (80). Thus, the results to date indicate that alteration and modification, which both affect RPase structure, are not essential elements in gene selectivity.

(iii) **De-alteration.** Horvitz (80) postulated that a prereplicative T4 enzyme removed ADP-ribosyl groups added by the alteration enzyme from the host RPase before the modification enzyme added ADP-ribosyl groups to the α subunits. This may explain the difference in the peptide patterns of altered and modified α subunits which both contain one ADP-ribose group; i.e., different arginine residues are rec-

ognized by the alteration and modification enzymes.

(iv) **Conversion of the β and β' subunits.** In the middle period of T4 infection (about 6 to 10 min after infection), both the β and β' subunits are converted to different forms, since the peptide pattern of these subunits differs significantly from that obtained with subunits from uninfected cells. The β differs in *his*- and *trp*-containing peptides, and the β' contains one additional *tyr*-positive peptide and a different *his*-containing peptide (174, 179). The peptide patterns of uninfected β and β' subunits differ sufficiently in that they exclude the possibility that β' is converted to β by loss of a low-molecular-weight polypeptide.

In the late period of infection (10 to 15 min after infection), a modification in the β' subunit was observed. The late β' subunit had a greater net negative charge as compared with β' from uninfected and early-infected RPase (205).

(v) **New RPase-associated polypeptides.** An analysis of RPase from the middle stage of infection (about 6 to 10 min after infection) revealed the presence of four polypeptides associated with the core with molecular weights of 22,000, 15,000, 12,000, and 10,000 (188, 189). T4 gene 55 and gene 33 code for the 22,000- (157, 189) and 12,000- (189) dalton subunits, respectively. These polypeptides are held tightly to the core and are not removed as readily as the sigma factor (189). They are not found associated with the core until after 5 min of infection. Since genes 55 and 33 are essential for the occurrence of late transcription, the association of their products with RPase core is highly significant. The 15,000-dalton subunit appears to be the product of the *alc* gene and may be involved in blocking host chromosome transcription (182a). The 10,000-dalton subunit may be an inhibitor of sigma activity (189a).

The various modifications in this section are summarized in Table 5.

Is there any correlation between these successive enzyme modifications and the sequential synthesis of different T4 RNA classes? A simple explanation would be that the successive modifications of the enzyme change the specificity successively and allow it to recognize specific promoters for the different classes of RNA. If this is indeed the case, there should be evidence for different forms of the enzyme transcribing specific RNA classes, e.g., IE, DE, QL, or L RNAs, either from the mature T4 DNA or from infected cell T4 DNA. Before consideration of each of the modifications and the evidence for the possibility of transcription specificity of each modified form, it should be noted that early in infection and throughout the infection cycle, the sigma factor is less firmly associated with the T4 cores (9). The T4 cores not only have less affinity for sigma factor, but also respond less efficiently to sigma factor in vitro. These observations indicate that early and subsequent modifications affect the affinity of the core with sigma factor. However, it is significant that these T4 cores can still be stimulated by sigma factor and that sigma factor can still be formed in infected cells (188, 189). It is possible that T4 cores interact with sigma factor in vivo in an effective manner.

What is the significance of the alteration, de-alteration, and modification functions in early and quasi-late periods of infection? As mentioned earlier in relationship to these modifications, these particular functions appear not to have any role in regulating gene specificity of the enzyme. Mutants that cannot carry out alteration or modification have been found which still express all necessary genes for normal phage replication (80). During the early period of infection, the IE and some DE RNA can be synthesized by cells that were treated with chloramphenicol before infection.

This chloramphenicol effect in vivo is complex and can be explained in at least the follow-

TABLE 5. Summary of RPase modifications after T4 infection

Modification	Subunit affected	Period	Nature of modification	References
1. Alteration	$\alpha \rightarrow \alpha_A = \alpha_{ADP-R}$	Early	ADP-ribosylation	69, 165, 173
	$\beta \rightarrow \beta_A = \beta_{ADP-R}$	Early	ADP-ribosylation	
	$\beta' \rightarrow \beta'_A = \beta'_{ADP-R}$	Early	ADP-ribosylation	
	$\sigma \rightarrow \sigma_A = \sigma_{ADP-R}$	Early	ADP-ribosylation	
2. De-alteration	$\alpha_A, \sigma_A, \beta_A, \beta'_A$	Early	Removal of ADP-ribose	80
3. Modification	$\alpha \rightarrow \alpha_M = \alpha_{ADP-R}$	Middle	ADP-ribosylation	68, 165
4. Conversion	$\beta \rightarrow \beta_M$	Middle	Antigenic, peptide maps	174, 179
5. Conversion	$\beta' \rightarrow \beta'_M$	Middle	Antigenic, peptide maps	174, 179
6. New RPase-associated polypeptides	22,000	Middle	Association of peptides to the middle-period core	188, 189, 157
	15,000			
	12,000			
	10,000			
7. Conversion	$\beta'_M \rightarrow \beta'_L$	Late	Electrophoretic property	205

ing two ways: (i) chloramphenicol is having a polar effect on transcription from a p_E promoter that serves both IE and DE genes (218); (ii) chloramphenicol is preventing the synthesis of a T4-specific product that allows recognition of promoters for DE and later genes (204). Polarity-suppressing genes are unable to prevent the blockage by chloramphenicol of all DE-gene expression, which suggests that a simple polarity mechanism is not in effect (218). Recently, in vitro complementation studies have indicated that RPase from cells that had been restricted by chloramphenicol before infection could transcribe DE genes from T4 DNA obtained from cells that had not been restricted by chloramphenicol. This suggests that the state of the T4 DNA early after infection is modified and that this DNA modification may provide a more readily accessible promoter for DE genes (197). Thus it is possible to have a promoter for DE genes located between the IE and DE sites made accessible by some T4 factor that affects the structure of T4 DNA. Therefore, it appears that early modifications of RPase may not be necessary for expression of DE genes and that there are two modes of expressing the early genes: (i) transcription by *E. coli* RPase from a p_E promoter site that allows reading of both IE and DE sites; and (ii) transcription by the same enzyme of DE genes from p_{DE} promoters made accessible by structural modifications of the T4 DNA. Since the same enzyme can recognize p_E and p_{DE} promoters, these promoters may in fact be identical or very similar.

Although the α modification may not be necessary for expression of phage genes, the modification of the α subunit does have a profound effect on the efficiency of host gene transcription. The α subunit from T4 8-min enzyme was reassociated in vitro with β and β' subunits from normal uninfected cells. This reconstituted enzyme with modified α recognized the *lac*, *trp*, and SuIII tRNA genes some 10, 4, and 4 times less efficiently, respectively, than enzyme with normal α subunit (119). These results have two very important implications. First, the T4 modification of α subunit may be involved in inhibition of host transcription after infection, and second, the core subunit α may play a major role in promoter recognition. This is the first evidence for such a role for the α subunit and implies a greater role of the core in gene specificity than heretofore realized.

Are there RPase modifications that correlate with the expression of QL genes? During this middle period of infection, a number of modifications occur, including covalent modifications of the β and β' subunits and the synthesis of a

number of polypeptides that associate with the T4 core (Table 1). These enzyme changes take place between about 3 and 8 min after infection, at a time when QL or middle RNAs are synthesized and the transition from early- to late-RNA synthesis occurs. The expression of QL and L RNAs requires the synthesis of protein after phage infection (214). Is there any evidence that T4 polymerase isolated at about 5 to 10 min after infection specifically transcribes middle or late genes? Are there new initiation factors that direct T4 core to p_Q and p_L promoter sites?

Although earlier data had suggested the presence of stimulatory factors that directed T4 polymerase to genetic sites which resulted in the synthesis of RNA that hybridized to the r or L-gene strands of T4 DNA (185), no purified T4 polymerase has been found that directs L-RNA synthesis in vitro. Part of this difficulty lies in the fact that L-RNA synthesis is either rigidly connected to or dependent on phage DNA replication (214). This makes it very difficult to say that the proper template has ever been used to assay for middle or late functions. Recent in vitro complementation studies have in fact indicated that p_Q promoters are recognized only after a T4-induced modification of infecting T4 DNA (197).

The recognition of the need for expression of genes 55 and 33 before L-RNA synthesis and the identification of the products of genes 55 and 33 as part of the late T4 polymerase suggested strongly that they played some role in L-gene specificity. However, when these enzymes were tested in vitro with or without added sigma factor, a high degree of symmetrical transcription of L genes occurred (189), and also much of any asymmetrical synthesis that occurred was from the early genes. It is possible that the proper template, if it is replicating DNA, may be very difficult to obtain for testing L-RNA synthesis in vitro. T4 polymerase also differs from *E. coli* polymerase in that it acts less efficiently on T4 DNA, is stimulated to a lesser degree by sigma factor, and has a different KCl optimum (189).

From the analysis of the available data, it is possible to state that the *E. coli* holoenzyme or altered T4 polymerase has specificity for the IE and some of the DE genes. T4 products coded by the early genes subsequently direct the enzyme to transcribe QL or middle genes. At least some of these genes are transcribed by the enzyme that recognized the promoter of the early class of genes. The T4 factor involved in QL-gene transcription appears to modify the T4 DNA template and not the RPase structure per se.

The modification of the T4 template makes p_0 promoters accessible to the enzyme. The transcription of L genes requires the products of genes 33 and 55 and the proper template that has L-gene promoters accessible to the enzyme. The fact that α modification is not involved in positive control of enzyme activity indicates that caution must be taken in interpreting structural changes in RPase in relation to its functional changes. Thus to date it is not possible to state unequivocally that RPase modifications play a role in gene selection in T4 infection.

T7 Phage Development

T7 is a virulent phage for *E. coli* and contains a linear double-stranded DNA of molecular weight 26×10^6 (45). From various studies (71, 82, 137, 183, 190, 195) it was demonstrated that a correlation existed between the set of genes expressed early after phage infection with the left end of the DNA and that the two sets of genes expressed later were mapped sequentially to the right of the early genes. By convention, the chromosome is divided from 0% at the left end to 100% to the right end (1% is approximately 370 base pairs). The DNA codes for approximately 30 proteins (191) with molecular weights ranging from 7,000 to 150,000. The genes are numbered from left to right on the DNA from 0.3 to 19.

During T7 infection and replication in *E. coli*, three classes of proteins have been identified that are synthesized according to a characteristic time course. Class I proteins are made between 4 and 10 min after infection, class II proteins are made between 6 and 15 min, and class III proteins are made between 7 and 30 min or lysis (190). The genes coding for class I proteins are early genes and are located at the left-most 20% of T7 DNA and include genes 0.3 to 1.3. The class II and class III proteins are specified by genes 1.7 to 6 and by genes 7 to 19, respectively. All transcription of T7 DNA is from left to right relative to the genetic map (190).

The five early genes 0.3, 0.7, 1, 1.1, and 1.3 are transcribed by the host RPase holoenzyme which recognizes three closely spaced early promoter sites near positions 0.5, 1, and 1.5%, i.e., at the left end of the genetic map (15, 24, 35, 36, 50, 129, 138). The RNA made from the promoters at 0.5 and 1.5% have pppA at their 5' end, whereas the RNA from the middle promoter at 1% is initiated with pppG (24, 50). Sequence analysis and other earlier studies (35, 36, 151, 152) have shown that the base sequence in early-gene promoter regions are similar but

distinct (Fig. 2; 152). The base sequences are within the promoter sequence spectrum recognized by *E. coli* holoenzyme. The holoenzyme transcribes the early genes by starting at one of the three possible early promoters and continues to transcribe the genes into a polycistronic mRNA. Four individual early mRNA's are derived from this early precursor RNA, which is cleaved at specific points by ribonuclease III (51). The holoenzyme recognizes a normal transcription termination site located at about 20% of the genetic map and does not transcribe the late genes of T7 that are located to the right of the early genes. Therefore transcription initiated at one of the early promoters is terminated at a single termination site. In vitro, the termination factor rho is not required for termination of early-gene transcription (25, 50).

How is the switch made from transcription of early to late genes? Does host RPase have any role in transcription of late genes? What is the mechanism for regulating late-T7 gene transcription? It has been shown very clearly that a T7-specific RPase is involved in late-gene transcription (23, 139). This T7 RPase is a product of one of the early genes, gene 1, and appears 4 to 6 min after infection. It is distinct from the host RPase holoenzyme in structure and specificity, is comprised of a single polypeptide with a molecular weight of about 110,000, and has appreciable activity only on T7, T3, and salmon sperm DNAs. It has very little or no activity on DNA from *E. coli*, T2, T5, λ , β 3, β 22, SP50, and P2 (25). It is also not inhibited by rifampin or streptolydigin (26).

The T7 RPase in the absence of any other protein factors is capable of transcribing all the late genes, genes 1.3 to 19. In vitro, seven major RNA species are synthesized (70, 71, 137). The promoter and terminator sites for these RNA species are listed in Table 6. Seven promoter sites and two terminator sites have been located, thus indicating that common terminator sites can be used. All seven promoter sites are recognized with equal efficiency by the T7 RPase, suggesting that they are very similar or perhaps identical. The rate of transcription by T7 RPase is also about five times greater than that by *E. coli* RPase (250 nucleotides/s versus 50 nucleotides/s) (71).

The high template specificity of T7 RPase suggests that the T7 DNA contains specific promoter sites that are not recognized by *E. coli* RPase. Thus the T7 promoter sequences fall outside of the promoter spectrum recognized by *E. coli* RPase. The T7 promoter may be rich in cytosine and thymidine residues, since single-stranded poly[d(C)] and poly[d(T)] and double-

TABLE 6. Properties of T7 RNA species and their promoter and terminator sites^a

RNA	Mol wt ($\times 10^6$)	Promoter site (%)	Terminator site (%)
I	5.50	56	99
II	4.50	64	99
IIIa	2.00	Unmapped	
IIIb	2.00	83	99
IV	0.84	46	53
V	0.44	49	53
VI	0.22	97	99

^a From references 70 and 137.

stranded $d(I)_n \cdot d(C)_n$ and $d(G)_n \cdot d(C)_n$ were active as templates (25).

In this and the related case with T3 phage (7), the transcription of developmental genes requires the synthesis of a new RPase with a specificity for distinctly new promoter sites. Interestingly, no factor similar to sigma factor was required for T7 RPase activity, indicating that the information for recognizing promoter sites and initiating transcription can be built into a single polypeptide chain.

A modification of *E. coli* host polymerase does occur after infection (220). A product of early gene 0.7 appears to be involved in the phosphorylation of the subunits of *E. coli* holoenzyme (155, 220). The activity of this kinase gene is not essential for subsequent development of the phage, and therefore it is highly unlikely that it is involved directly in the switching process from transcription of early to late genes, i.e., in recognition of late promoter sites. It may, however, be involved in controlling the further transcription of host genes and the early T7 genes by the host holoenzyme, since synthesis of host RNA is discontinued at 3 min after infection and the rate of synthesis of the early genes is decreased to zero at 8 to 12 min after infection. The kinase phosphorylates the β' subunit in particular, but also phosphorylates the other RPase subunits to a lesser degree as well as a number of other host proteins. Since the β' subunit is involved in DNA binding, it is particularly intriguing but not surprising that it may be modified during control of host RPase activity, i.e., to decrease the host RPase binding affinity or promoter recognition by steric, chemical, or charge hindrance. The T7 protein kinase function appears to be necessary for efficient propagation of phage in poor media or at high temperatures and appears to affect the accumulation of late proteins under these conditions (79a).

This particular example with T7 phage development is a classic example of synthesis of a

completely new RPase for the recognition of distinct and specific promoter sites for developmental genes. This varies considerably with the case discussed previously with T4 phage infection, which utilized the host core enzyme throughout its infection cycle.

Lambda Phage Development

A number of excellent recent reviews are available on the control of gene expression in lambda phage development (77, 153, 209). Comments will be limited to a discussion of sequential transcription of lambda phage genes during the lytic response and the data concerning factors that may control RPase specificity during sequential transcription.

The lambda genes can be divided into three groups, depending on the functions required for their transcription (77). Group 1 genes require no phage products for transcription and include genes *N* and *cro* (Fig. 1). Group 2 genes require λ N protein and include genes *int*–*cIII* and *cII*–*Q*. Group 3 genes require λ Q protein and include genes *SRA*–*J*. The sequence of transcription follows the group numbers in increasing order, with group 1 and group 2 genes called early genes and the group 3 genes called late genes. Group 1 genes can be transcribed by *E. coli* RPase holoenzyme in vitro, and their expression is inhibited by rifampin, but not by the presence of chloramphenicol in vivo. The *N* gene is transcribed leftward and therefore from the l DNA strand, and the *cro* gene is transcribed rightward from the r strand. The *N* gene is transcribed from the promoter p_L , and the *cro* gene is transcribed from the promoter p_R . The expression of the p_L promoter is controlled by two tandem operator regions, o_L1 and o_L2 (122–124), which bind lambda repressor coded by the *cI* gene. A set of three tandem operator sequences regulates the p_R promoter, and these are designated o_R1 , o_R2 , and o_R3 (150, 208). The sequences for these operator sites are listed in Table 1.

During the lytic cycle, the repressors are removed from the operator sites, allowing *E. coli* holoenzyme to bind to p_R and p_L . This results in the synthesis of the products from genes *N* and *cro*. The product of gene *N* has been shown to be essential for the transcription of the early genes *int*–*cIII* and *cII*–*Q*. The *N* gene product is a protein which has been found to be associated with *E. coli* RPase holoenzyme (55). The size of the RNA polymerase-bound protein is 11,600 daltons, although it may be derived from a precursor of 14,500 daltons. This modified RPase molecule containing the *N* gene product recognizes the same promoters as the unmodi-

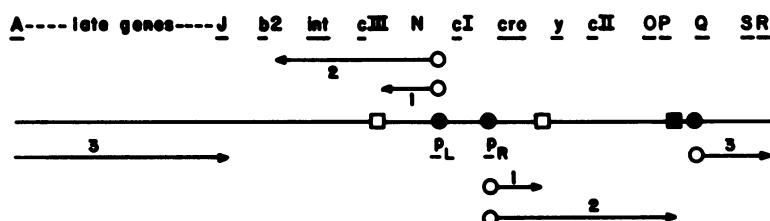


FIG. 1. Genetic and transcription map of λ bacteriophage (from reference 77). Symbols: ●, Promoter sites; ○, early transcription initiation sites; □, early termination sites (group 1 genes); ■, early termination sites (group 2 genes).

fied RPase molecule, i.e., p_R and p_L , and now is able to transcribe the chromosome beyond the termination sites for *cro* and *N* genes, respectively (see Fig. 1). The *N* protein thus acts as an antiterminator factor (1, 55, 77). Since the same promoter sites are recognized by the RPase with and without the *N* gene product, it indicates that the *N* gene product is not altering the template specificity of the *E. coli* RPase.

The *Q* gene product is required for transcription of the group 3 or late genes (77). The mechanism of *Q* gene product stimulation of late transcription is not known. However, a high-level synthesis of late gene products requires a promoter between genes *Q* and *S* (Fig. 1). After the RPase binds to this promoter, it is capable of transcribing genes *SR* and *A* through *J*. Some data indicate that *Q* gene product may function as an antiterminator (164). Does the recognition of the promoter between genes *Q* and *S* by the *E. coli* RPase require some change in enzyme specificity; i.e., is the promoter sequence outside the normal promoter spectrum recognized by the *E. coli* enzyme? More biochemical analysis is required of the RPase from late stages of induction to answer this question.

B. subtilis Phage SP01 Development

The RPase of *B. subtilis* has been studied extensively, and recent analyses have revealed that its structure is quite complex. Besides the normal procaryotic core subunits (α , β , and β') and σ factor, the normal vegetative-cell enzyme contains at least three other core-associated subunits of relatively low molecular weight (Table 7; 47, 148, 212). The exact functions of these smaller subunits, ω_1 , ω_2 , and δ , are still unknown, although the δ subunit may be involved in increasing the specificity of transcription on certain strands of DNA (114, 148).

After infection of *B. subtilis* by phage SP01, which contains hydroxymethyluracil for thymine in its DNA, a sequential appearance of six types of mRNA occurs (60, 64). The early class (*e*, *em*) appears within 1 min of infection independent of viral protein synthesis; the middle

TABLE 7. Subunits of RPase from *B. subtilis* vegetative cells^a

Subunit	Mol wt
β'	160,000
β	150,000
σ	55,000
α	45,000
δ	21,000
ω_1	11,000
ω_2	9,500

^a From references 4, 5, 49, 63, 115, 118, and 182.

class (*m*, *m₁*, *m₂*) first appears between 4 and 10 min of infection before DNA replication; the late class (*l*) is synthesized from about 11 min after the onset of SP01 DNA replication. The early genes are transcribed by the host holoenzyme. The synthesis of the middle class of RNA transcripts is dependent on the expression of gene 28 of the SP01 genome (60, 64), which codes for a protein with a molecular weight of 26,000 to 28,000 (59).

RPase isolated during the middle period of infection, e.g., 10 min after infection, contains several polypeptides in addition to the vegetative-cell core subunits (Table 8). Geiduschek and his colleagues (47, 49) have reported the presence of two subunits with molecular weights of 28,000 and 13,000, whereas Pero and her colleagues (57, 148) have found three polypeptides with molecular weights of 26,000, 24,000, and 13,500. The stoichiometric relationship of the 28,000- and 13,000-dalton subunits to the core on the average was 1.4 and 1.5, respectively (47); however, the ratio varied from 0.6 to 2.4 for each of the subunits. Although an exact stoichiometric relationship was not stated for the three subunits found by Pero et al. (148), it appeared that the 26,000-subunit was present at close to 1, whereas only very small amounts of the 24,000- and 13,500-subunits were present, i.e., much less than 1:1 with the core.

Recent studies indicate that the 28,000-dalton subunit, which had been designated as P^{28} (47) and ν^{28} (49), and the 26,000-dalton subunit, also designated as subunit IV (57), are the same

TABLE 8. *RPase subunit composition during SP01 infection of B. subtilis*^a

SP01 genes read	Subunit composition
Early	$\alpha_2, \beta, \beta', \delta, \omega_1, \omega_2, \sigma$
Middle	$\alpha_2, \beta, \beta', \delta, \omega_1, \omega_2, 28K^b$
Late	$\alpha_2, \beta, \beta', \delta, \omega_1, \omega_2, 24K, 13K$

^a From references 49, 57, 58, and 202.^b K = 1,000 daltons.

subunit (114). The SP01-modified RPase core containing this subunit is able to transcribe specifically in vitro the middle genes of SP01 (46, 47, 148). Hybrid competition studies have indicated that this enzyme transcribes the middle genes from the H strand of DNA (47, 148). Moreover, it does not transcribe the early genes to any degree. No sigma factor is required for this specific transcription. Reconstitution studies have shown that the addition of this subunit to the core converts the core to the enzyme that can specifically transcribe middle genes (47, 148). These studies with the middle enzyme of SP01 demonstrated that a polypeptide other than sigma factor could cause the RPase to transcribe a set of genes not recognized by the holoenzyme. In fact, kinetic data obtained with rifampin inhibition studies indicate that the SP01 middle enzyme forms an "open" complex with DNA, i.e., the tight binding complex (48). Analysis of the core subunits α , β , and β' after phage infection have not revealed any changes in their structure; i.e., no covalent modifications have been detected. Thus the only modification in structure appears to be the presence of the 26,000- to 28,000-dalton subunit, the product of gene 28. Two interpretations of its action are possible: (i) It allows the enzyme to recognize promoters for middle genes that are very different from early-gene promoters, and it serves as a positive control element; (ii) it allows the enzyme to recognize middle-gene promoters that are not different from early-gene promoters, but allows the enzyme to read through a very early termination signal in the middle genes. The fact that early genes are not transcribed in vitro suggests that the modified enzyme no longer recognizes early promoters and makes the second interpretation less likely. The acceptance of the first interpretation suggests that the early-SP01 gene promoters and the *B. subtilis* gene promoters differ from those for the middle and late genes of SP01; i.e., they belong to spectra of promoters that do not overlap.

It has been shown recently that the ν^{28} fraction (49) contains not only the 28,000 (or 26,000)-dalton subunit but also a 24,000-dalton

subunit (114). Thus both Geiduschek's and Pero's data now show the presence of three major phage-coded subunits that can associate with the host core: 26,000 to 28,000, 24,000, and 13,000 to 13,500. Pero and her colleagues have recently shown that the enzyme containing the subunits of 24,000 (termed V) and 13,500 (termed VI) daltons preferentially copy late genes in vitro (202). Moreover, these polypeptides are derived from the SP01 regulatory genes 34 and 33 (57), which control late-gene expression in vivo (60, 114). Pero and her colleagues (202), by reconstitution studies, have shown that the presence of V, VI, and δ factor causes selective transcription from the H strand of SP01 DNA of the late genes. The absence of any one of the polypeptides caused the synthesis of anti-mRNA, indicating that symmetric transcription occurred in the absence of any of the polypeptides. Since the absence of the host factor δ also resulted in a large proportion of anti-mRNA synthesis, its role may involve proper strand selection during transcription. The absence of δ factor from the enzyme can perhaps be compensated in vitro by high salt concentrations in the reaction mixture (114).

The synergistic effect of the products from genes 34 and 33 is in apparent agreement with earlier studies which showed that a mutation in either gene resulted in a blockage of late-RNA synthesis in vivo (60).

Thus, although these studies do not show that all middle or late genes are being transcribed by these modified RPase, no other modifications of the core subunits or other factors have been found that are required for selective transcription of these genes. It is of interest that each form of the enzyme is selective for a certain set of genes, although the hybridization studies do not rule out completely that the middle or late enzymes may not have low activity on the other sets of genes. In essence, however, since these core-associated polypeptides do not have any as yet defined antiterminator role, it appears that the promoter recognition specificity of the core enzyme can be modified sufficiently by these polypeptides to recognize a new spectrum of promoters.

Similar data concerning the sequential transcription of genes and the modification of RPase have been obtained with phage SP82 (100, 186, 187, 212).

B. subtilis Sporulation

A number of biochemical studies have indicated that differential gene expression occurs during sporulation in *Bacillus* species (44, 74). DNA-RNA hybrid competition studies with

RNA from sporulation cells have revealed a sequential appearance of mRNA's that are not found during vegetative growth (2, 40, 43, 193, 215). These analyses also showed that most vegetative genes are not "turned off" during sporulation and that sporulation genes are actually "turned on" in addition to the vegetative genes (40, 91, 193). Therefore, sporulation is more complex than the vegetative growth phase.

The initial sporulation events begin during the early stationary phase of growth. Release from catabolite repression is necessary but not sufficient for sporulation to occur (97). Is there any evidence that the RPase function or structure is changing during sporulation to explain the derepression of sporulation genes? Studies have revealed that rifampin-sensitive cells remain sensitive throughout sporulation, indicating that the same RPase core is used throughout sporulation, or that if a new RPase is made, it is also rifampin sensitive (108). Analogous results were obtained with a rifampin-resistant temperature-sensitive RPase mutant (108). These studies suggest that the β subunit of the RPase core is functioning throughout sporulation, but do not indicate that a vegetative-type core containing this subunit is the only type present. Continuous transcription also occurs during sporulation (107, 108).

Early studies of Yehle and Doi (217) indicated that certain phages were able to replicate successfully in sporulating cells, whereas other phages injected their DNA but did not replicate. Subsequent studies of this phenomenon have shown that the degree of ability for replication in sporulating cells may depend on the complexity of the phage (86, 93). One interpretation of this phenomenon has been that the phage genome promoters are recognized by vegetative-cell holoenzyme but not by sporulating-cell RPase (116) and that the lessened affinity of sigma factor to the core during sporulation may prevent transcription of the phage genes (178, 201). Other data and interpretations suggest that the sporulating cell is able to replicate phage but at a reduced rate and that the sporulating-cell RPase is indeed able to recognize phage promoter sites (93). In any case, these studies, in addition to the earlier evidence concerning sporulation-specific gene products, suggested that RPase specificity was modified during the sporulation process.

Two sets of data with RPase mutants (Rif^r) that are temperature sensitive only during sporulation suggest that the structural integrity of the wild-type core is essential for sporulation; i.e., these Rif^r mutants can grow at the same rate as wild type at the nonpermissive

temperature, but stop sporulating at different stages, depending on the mutation at the nonpermissive temperature (106, 194). These observations suggest that the RPase core may have to interact with a "modifying factor" during sporulation and that the mutant RPase cannot interact properly because of its improper conformation at the nonpermissive temperature. According to this idea, the "modifying factor" could cause covalent modification of one or more of the subunits of the core or, if the modifying factor is a regulatory polypeptide, it could associate with the core. Both of these types of modification could result in an RPase with new promoter specificity.

To date no covalent modification of any of the core subunits has been observed with *B. subtilis* RPase. Earlier results showing a smaller β subunit in sporulation RPase (109, 115) have been found to be in error due to artifacts of enzyme isolation (63, 111, 145). When protease activity was inhibited during enzyme purification, no change in the molecular weight of β was observed.

When RPase was isolated under conditions in which protease activity was inhibited, several different forms of RPase were observed. Doi and his colleagues (61, 63) have observed the presence of two forms of enzyme in sporulating stage III cells. One form (enzyme I) is similar to or identical with vegetative holoenzyme ($\alpha_2\beta\beta'\sigma$), and the other is a new form of enzyme (enzyme II) with the composition $\alpha_2\beta\beta'\delta^1$, where δ^1 is a sporulation-specific peptide with a molecular weight of 27,000 (61, 63). This second form did not have sigma factor associated with it. At stage IV of sporulation another form of the enzyme (enzyme III), with the composition $\alpha_2\beta\beta'\delta^2$, was detected in addition to enzymes I and II. The δ^2 subunit had a molecular weight of around 20,000. Again enzyme III did not have σ associated with it. It is unlikely that δ^2 is the equivalent of the δ factor (148) of 21,000 daltons found associated with the vegetative core, since the purification procedures used to obtain δ^1 and δ^2 did not yield the vegetative δ factor. Enzymes II and III differ from enzyme I by their greater affinity to the DNA-cellulose column, by their higher specific activity on various DNA templates, and by the tighter association of the δ^1 and δ^2 factors to the RPase core relative to sigma factor to core in enzyme I. The continued presence of enzyme I during sporulation is consistent with the continued synthesis of "vegetative" mRNA during sporulation (40, 91, 193).

By use of slightly different procedures for isolation of RPase, Losick and his colleagues

(72, 110) have reported the isolation of RPase core with the usual composition of $\alpha_2\beta\beta'$ plus an enzyme III with core plus two additional polypeptides, P^{85} and P^{27} , with molecular weights of 85,000 and 27,000, respectively. The 85,000-dalton subunit is apparently equivalent to their previously mentioned 70,000-dalton, core-associated subunit (72). The stoichiometric relationship of the P^{85} and P^{27} subunits to the core was 0.5 to 1.0.

The major differences between the data of the two groups are the apparent absence of holoenzyme in Losick's preparation and the presence of the P^{85} polypeptide in his enzyme III. Fukuda and Doi (61) did not find any 85,000-dalton polypeptide associated with the core of their enzymes II and III. The two groups do agree on the presence of δ^1 (i.e., P^{27}) in stage IV cells. Neither group reported the presence of the 21,000-dalton vegetative δ factor (148) in their sporulation RPase. A much more complex picture is emerging with studies on RPase from *Bacillus thuringiensis* (96, 97).

Although these two groups have shown that RPase structure is modified during sporulation, neither group has shown that these modified RPases can recognize sporulation-specific promoters. Preliminary studies by Nakayama and Doi (unpublished observations) have shown that netropsin, an antibiotic that binds to A-T-rich regions of DNA (210), inhibits sporulation enzyme II much more effectively than vegetative holoenzyme with *B. subtilis* DNA as template; these results suggest that vegetative holoenzyme and sporulation enzyme II may recognize a different spectrum of promoter sequences. Sporulation RPase may recognize promoters that are much more A-T rich than promoters for vegetative genes. Since the only apparent difference between the holoenzyme and enzyme II is the presence of sigma factor in one and the δ^1 factor in the other, the δ^1 factor may play some role in promoter recognition. Although a considerable amount of effort has been taken to clarify the situation with *B. subtilis* sporulation RPase, the problem has been compounded by the presence of protease activity in the extracts and the different methods of purification used by the various investigators. Recent methods of removing protease activity from extracts by protease affinity columns (136) and the use of suitable enzyme purification procedures are leading to more in vivo-like RPase enzymes.

CONCLUSIONS

A pattern has emerged which indicates that selective gene transcription occurs through four general types of mechanisms:

(i) New phage-specific RPases have been identified with T7 and T3 phages that infect *E. coli*. These RPases recognize phage-specific promoters that fall outside of the normal *E. coli* spectrum of promoter sequences. They do not need sigma factor for activity, and their specificity is such that they do not recognize *E. coli* promoters. The fact that they consist of a single polypeptide indicates that the recognition, binding, and initiation functions of RPase can be carried out by a relatively simple polymerase enzyme. It is possible that the phage-specific RPases for PBS2 (27) and gh-1 (203) are also similar in nature to the T7 RPase in recognizing promoter sequences different from their hosts' promoter sites.

(ii) The second type of mechanism evident in procaryotic cells is the modification of an existent RPase molecule to change its promoter recognition specificity. This is evident from the data from the *B. subtilis* phages SP01 (47, 148) and SP82 (212). After infection of the host with these phages, a phage-specified polypeptide associates with the host's core enzyme to form an enzyme with altered template specificity. The enzyme is modified in such a manner that it will no longer recognize the host promoter sites.

(iii) The third means for differentially transcribing genes is best exemplified by the development of the temperate phage for *E. coli*, λ . In the case of λ , a phage-specified *N* gene protein (55) interacts with the host RPase and changes its termination but not its initiation specificity. Therefore, the selection of subsequent developmental genes is the result of antitermination activity. The modified RPase probably recognizes promoters very similar to or identical with the host's promoters.

(iv) The fourth general class of regulatory mechanism utilizes a number of proteins that interact with the promoter region and either inhibit or facilitate the initiation of gene transcription. Only the mechanisms of the repressor and the CAP protein have been well characterized; however, a number of other proteins affecting transcription have been isolated. In these cases, gene selection is dependent on factors other than the RPase itself that recognizes the general spectrum of promoters.

Thus, a number of complementary mechanisms for gene selection have evolved in procaryotic cells. Two of these mechanisms show that RPase does play a direct role in gene selection. One must keep in mind that this discussion has been limited to one narrow aspect of the role of RPase in gene selection and that this simplistic view is being changed rapidly. A recent review by Travers (206) indicates that the complex procaryotic holoenzyme interacts with

a number of cellular metabolites that regulate coupled translation-transcription processes. The next few years should bring forth a number of discoveries that will explain why the procaryotic holoenzyme is such a complex multimeric enzyme.

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